

P023 Development of transcription-inactive p53-fusion proteins (TIFp53) as a high-throughput screen system to identify compounds stabilising and promoting p53 tumour suppressor activity

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The successful use of proteasome inhibitors, in anti-cancer therapy encouraged the development of alternative drugs acting at various levels of the Ubiquitin-Proteasome System (UPS). One of the most specific intra-molecular interactions that can be considered as targets for drug development is the Ubiquitin ligase: substrate interface. For this reason we have developed a drug screening system called TIFp53 (Transcription Inactive p53-Fusion proteins), to be used in high-throughput screens. The system contains all p53 sequences required for an optimal degradation of the tumour suppressor p53 mediated by Mdm2 during DNA damage. The DNA binding domain appears to be dispensable in this process, and removal of this domain therefore abrogates the transcription-dependent effects of the negative feedback of the system. Taking advantage of these chimeric proteins we have developed a system based on the Luciferase-p53 fusion (Luc-p53), which is co-expressed together with Mdm2 in the p53 knock out cells H1299. After validation using transient transfections, the expression of Luc-p53 was stabilised in H1299 cells together with a tet-on inducible vector encoding the human version of Mdm2 to facilitate the screening of large collections of molecules. In this way a screening cell line was developed to facilitate drug identification.